

A MICROMETHOD FOR THE DETERMINATION OF ARGININE

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Micromethods for the determination of arginine based on the use of the Sakaguchi reagent have been described (1-4). This reagent gives a strong color with glycoeyamine, arginine, and other monosubstituted guanidine derivatives.

In a previous communication (5) a method for the determination of glycoeyamine was described based on the Sakaguchi reaction and the quantitative separation of glycoeyamine from arginine by selective adsorption of the arginine on permutit. In the method outlined below the separated arginine is eluted from the permutit and determined independently.

A number of common non-chromogenic substances such as ammonia, urea, histidine, and creatine reduce the color obtained with the Sakaguchi reagent. A change in the usual order of addition of the color reagents similar to that previously described (5) decreases the interference by these compounds.

Reagents and Apparatus—

3 per cent sodium chloride.

0.3 per cent sodium chloride.

Permutit according to Folin. Permutit can be regenerated after use by allowing 3 per cent sodium chloride to percolate through and then washing with distilled water until chloride-free. This is most conveniently done in large batches on a Buchner funnel.

10 per cent urea in water.

0.2 per cent naphthol in absolute alcohol, diluted with 4 volumes of the 10 per cent urea solution before use.

Hypobromite solution. 0.66 ml. of liquid bromine are added

to 100 ml. of 5 per cent sodium hydroxide. Since this solution deteriorates, it should not be kept for more than 1 or 2 days, and is best kept in a refrigerator.

10 mg. per cent arginine in 0.1 N hydrochloric acid. Standard solutions are made on the day on which they are to be used, by diluting this stock solution with water.

Adsorption column. The permutit is contained in the stem of a glass funnel whose dimensions are: upper part 15 mm. external diameter, 100 mm. long; stem 7 mm. external diameter, 100 mm. long. The lower end of the stem is slightly constricted. A small amount of cotton is placed above the constriction. 0.9 gm. of permutit is poured in and tapped gently to settle the particles.

A 0.2 ml. micro pipette whose contents are delivered under pressure. The delivery time should be sufficiently short to insure rapid delivery and complete mixing (6).

Preparation of Solutions for Analysis—For complete separation of glycocyamine and arginine the salt concentration of the solution should not be over 0.5 per cent. If neither of these compounds is present in amounts over 2 mg. per cent, the salt concentration may be as high as 1 per cent. Urine is usually diluted 5 to 10 times with water. Blood filtrates may be prepared by deproteinizing according to Folin and Wu or by heat coagulation at pH 6 after 1:10 dilution with water. Tissue extracts are diluted to contain 1 gm. of tissue (fresh weight) in 40 ml. of suspension. The pH is adjusted to 6.0, and the suspension immersed in a boiling water bath for 10 minutes, cooled, and filtered. Analyses are carried out on the filtrates.

Procedure

5 ml. of the solution to be analyzed are passed through the permutit column and the small amount of glycocyamine remaining in the column is removed with 5 ml. of 0.3 per cent sodium chloride. The combined filtrate contains all the glycocyamine. A test-tube graduated at 10 ml. is now placed under the funnel, and the arginine is eluted by passing 10 ml. of 3 per cent sodium chloride through the column. The solution in the test-tube is made up to the 10 ml. mark and shaken.

A 2 ml. aliquot is taken for analysis. It is first cooled in an ice bath, then 0.5 ml. of the ice-cold naphthol-urea solution is added,

and after 2 minutes 0.2 ml. of ice-cold sodium hypobromite solution added by means of the micro pipette. This pipette is placed above the solution level and kept away from the sides of the test-tube to prevent contamination by urea in the solution. The color is simultaneously developed in a series of standard solutions containing 0, 0.25, 0.5, 1.0, and 2.0 mg. per cent of arginine.¹ After 20 minutes the color development is complete and remains stable for 2 hours if the solutions are kept in an ice bath. The tubes are shaken for a few seconds to remove excess gas, warmed by immersion in water at room temperature, and the intensity of

TABLE I
Elution of Arginine from Permutit Column

5 ml. of a 2 mg. per cent solution of arginine in 0.5 per cent sodium chloride were passed through the adsorption column followed by 5 ml. of 0.5 per cent sodium chloride. The arginine was then eluted by the salt solution as shown.

| Sodium chloride eluting solution | | Arginine recovered |
|----------------------------------|-----------------|--------------------|
| Amount | Concentration | |
| <i>ml.</i> | <i>per cent</i> | <i>per cent</i> |
| 10 | 0.5 | 0 |
| 5 | 1.0 | 2 |
| 10 | 1.0 | 40 |
| 5 | 2.0 | 60 |
| 10 | 2.0 | 100 |
| 10 | 3.0 | 100 |
| 10 | 5.0 | 100 |
| 5 | 5.0 | 88 |
| 10 | 10.0 | 88 |

the color measured in a spectrophotometer or a colorimeter, with light of approximately 0.525μ (yellow-green).

DISCUSSION

As shown in Table I, the complete removal of arginine from the permutit column depends on the amount as well as the concentration of salt solution used. Under the conditions described, the separation of glycocyamine and arginine is complete over a wide

¹ If an appropriate correction factor is applied, glycocyamine standards which are stable may be used.

range of concentrations (Table II). With concentrations of arginine of over 2 mg. per cent the color is too intense to be read and must be diluted before the color is developed. The blanks are yellow instead of colorless as in the procedures described in the literature.

In these older procedures a large excess of urea is added after a given interval to prevent destruction of the chromogenic compound by the hypobromite. During this interval other substances may compete for the hypobromite and reduce or completely inhibit the formation of color. The advantage of the procedure described

TABLE II

Limiting Concentrations of Glycoeyamine and of Arginine That Can Be Separated on Permutit Column

All values are concentrations in mg. per cent. The concentrations were those of the solutions passed through the permutit column. The final filtrates were diluted to bring the concentration to approximately 1 mg. per cent before the color was developed.

| Arginine added | Glycoeyamine added | Arginine found |
|----------------|--------------------|----------------|
| 0.0 | 2.0 | 0.0 |
| 0.0 | 2.5 | 0.0 |
| 0.0 | 5.0 | 0.0 |
| 0.0 | 10.0 | 0.0 |
| 1.0 | 0.0 | 1.0 |
| 2.0 | 0.0 | 2.0 |
| 5.0 | 0.0 | 5.0 |
| 10.0 | 0.0 | 9.6 |
| 20.0 | 0.0 | 18.4 |

above is that this interference is greatly reduced and for most purposes is negligible.

Table III shows the recovery of arginine added to urine, kidney extract, and blood.

Weber (2) has studied the influence of some non-chromogenic substances on color development. He found that 6 mg. per cent of ammonia, 12 mg. per cent of histidine dihydrochloride, 6 mg. per cent of tyrosine, 6 mg. per cent of tryptophane, 40 mg. per cent of creatine, or 160 mg. per cent of urea² "either prevents all color

² These are the concentrations in the final diluted filtrates.

formation or the color is so altered that even for qualitative purposes the test is worthless." With the method described in this communication, 60 mg. per cent of ammonia, 5 mg. per cent of histidine hydrochloride, 8 mg. per cent of tyrosine, 8 mg. per cent of tryptophane, 20 mg. per cent of creatine, and 2000 mg. per cent of urea are without influence on the recovery of arginine. If tryptophane and histidine are present in excess of the

TABLE III

Recovery of Arginine Added to Urine, Kidney Extract, and Blood

All the values are concentrations in the eluate measured in mg. per cent. The urine was diluted 1:4 with water. The kidney extract was made by grinding up 1 part by weight of kidney with 40 parts of 0.5 per cent sodium chloride, bringing the extract to pH 5.0, boiling 10 minutes, cooling, and filtering. The analysis was carried out on the protein-free filtrate. In both the kidney extract and blood, arginine was added before protein precipitation.

| Arginine added | Arginine | | | | | |
|----------------|----------|----------------------------------|----------------|----------------------------------|-------------------------|----------------------------------|
| | Urine | | Kidney extract | | Folin-Wu blood filtrate | |
| | Found | After subtraction of blank value | Found | After subtraction of blank value | Found | After subtraction of blank value |
| 0.0 | 0.13 | | 0.15 | | 0.19 | |
| | 0.13 | | 0.14 | | 0.20 | |
| 0.2 | 0.345 | 0.215 | | | 0.41 | 0.21 |
| | 0.34 | 0.210 | | | 0.43 | 0.22 |
| 0.5 | 0.61 | 0.48 | 0.65 | 0.505 | 0.68 | 0.48 |
| | 0.61 | 0.48 | 0.655 | 0.51 | 0.68 | 0.48 |
| 1.0 | 1.12 | 0.99 | 1.19 | 1.045 | 1.19 | 0.99 |
| | 1.13 | 1.00 | 1.19 | 1.045 | 1.19 | 0.99 |
| 1.5 | | | 1.64 | 1.50 | 1.67 | 1.47 |
| | | | 1.64 | 1.50 | 1.71 | 1.51 |

amount given, they are chromogenic, and, a further complication, histidine reduces the color given by the arginine present. In pregnancy urine the concentration of histidine may be as high as 160 mg. per cent (7) and the method is inapplicable as it stands. The method is satisfactory for normal urine, although the possible presence of other chromogens, such as methylguanine, must be borne in mind.

SUMMARY

A micromethod for the determination of arginine in biological fluids and tissue extracts is described. The advantages of this new method are that a complete separation of arginine from glycoeyamine is effected, and that it gives satisfactory results in the presence of common biological substances which interfere in the procedure of previous methods.

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